FLORAL STRUCTURE, HYBRIDIZATION AND EVOLUTIONARY RELATIONSHIP OF TWO SPECIES OF MIMULUS[1]

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Floral characters of angiosperms have long been recognized as a useful guide in plant systematics. The diverse floral structures represent results of long periods of natural selection and often reflect a close relationship with breeding systems of plant species (Stebbins, 1970). Furthermore, they also play an important role in reducing interspecific crossing (Grant & Grant, 1965). Therefore, studies of floral characters in relation to breeding systems and ecological adaptation of plant species would contribute toward the understanding of processes of plant speciation.

Mimulus guttatus D. C. (Yellow monkey flower) and *M. nasutus* Green (Fig. 1) are two closely related species abundantly occurring in moist areas in California (Grant, 1924). The flowers of both species have yellow corollas with variable numbers and sizes of red blotches (Abrams, 1951). *Mimulus guttatus* is a perennial, self-compatible, but normally outcrossing species (Kiang, 1972), while *M. nasutus* is an annual and predominantly autogamous species. Since both species have similar geographical distribution, yet adopt distinctly different growth and breeding habits, they are desirable for the study of phylogenetic relationship by examining floral morphologies, hybridization and phenology in relation to their ecological adaptation. This paper reports the floral structure, breeding systems, hybridization and the possible evolutionary relationship of the two species. The studies indicate that the two species are phylogenetically closely related and *M. nasutus* is probably a daughter species of *M. guttatus*.

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M. guttatus

MATERIALS AND EXPERIMENTAL PROCEDURE

Populations of *Mimulus guttatus* and *M. nasutus* were established in the greenhouse from seeds collected from 10 randomly chosen plants from a single population of each species. The *M. guttatus* population was growing in granitic sandy soil along shallow channels of a creek about 800 m west of the entrance of Eureka State Park, California (120.70°W., 39.37°N.; elevation about 1820 m), while the *M. nasutus* population was in a roadside ditch watered by a small seep in the road cut of State Highway 70, Feather River Canyon near Rock Creek, California (121.28°W., 39.98°N.; elevation about 610 m).

Crosses between the two species were made on twenty plants of each species. In order to avoid contamination caused by the precocious dehiscence of anthers of *Mimulus nasutus,* emasculation had to be performed on the flower buds two or three days before they opened.

Since flowers of both species exhibit some degree of intraplant variation, only the third or the fourth node flowers on main stems were used for measuring the size of floral parts as well as estimating pollen viability by staining pollen with lactophenol aniline blue. The number of seeds from the mature capsules was counted, and the seed viability tested by germination. Total fertility and productivity of the two species and the hybrids were assessed. Chromosome counts were made from pollen mother cells following the techniques used by Mia *et al.* (1964). Both species were found to be diploid with n = 14.

RESULTS AND DISCUSSION

1. Floral characteristics and breeding system.

Viable seeds were harvested from all capsules on which interspecific pollination was attempted. *Mimulus guttatus*

Fig. 1. *Mimulus guttatus* (left) and *M. nasutus* (right) grown in the same pot (4″) for about 8 weeks in the greenhouse. Many capsules can be seen on *M. nasutus,* while *M. guttatus* is just coming into bloom.

TABLE 1. The length of the floral organs and the ratio of stamen to pistil (in mm).

		Calyx	Corolla	Ovary	Style	Pistil	Stamen[1]	Stamen/ Pistil
M. nasutus	Mean	11.4	10.5	3.2	4.0	7.2	7.2	1.09
N = 74	CV	17.7	23.9	15.6	20.8	14.8	13.1	5.4
M. guttatus	Mean	9.4	26.5	4.6	12.1	16.8	12.9	.76
N = 45	CV	16.1	11.4	12.5	6.0	5.8	9.7	8.3
F_1	Mean	11.0	26.0	4.5	11.4	16.0	14.0	.86
N = 62	CV	13.1	11.1	11.7	7.6	6.6	7.5	6.2
F_2	Mean	11.1	21.0	3.9	9.6	13.5	11.4	.85
N = 58	CV	12.8	19.7	16.8	12.5	12.0	10.9	9.5
F_1 × *M. nasutus*	Mean	12.3	17.9	3.5	7.8	11.3	10.0	.90
N = 21	CV	8.2	28.7	16.8	25.2	19.7	14.5	10.5
F_1 × *M. guttatus*	Mean	10.6	23.6	4.2	10.5	14.8	12.0	.81
	CV	19.4	11.7	16.4	12.1	10.8	13.0	9.4

[1]Only long pair of stamen was measured.

N — Number of observation.

CV — Coefficient of variation (standard deviation erpressed as a percentage of the mean).

has smaller calyces and larger corollas than *M. nasutus* (Table 1). Floral parts of *M. guttatus* are relatively stable with respect to size. The stability of the floral characteristics is essential for the insect-pollinated plant species in achieving successful seed production. Any drastic change in floral parts may result in failure of pollination by pollinators. Contrary to the rather stable flower size of *M. guttatus, M. nasutus* exhibits morphological plasticity of the flower, particularly corolla size. Flowers are progressively reduced in size on a plant, and after ten nodes or so on the main stem, flowers often become so small as to be inconspicuous. Since *M. nasutus* is predominantly self-pollinated, there is no necessity to attract insect visitors by having large, showy flowers.

Both species have two pairs of stamens, one short and one long, in each flower. The two pairs of stamens in *Mimulus nasutus* are more or less similar in length and the anthers are clustered around the stigma. The bilabiate stigma, small and insensitive, always remains open, even in the flower bud. Thus, the structural arrangement of floral parts of *M. nasutus* greatly facilitates successful self-pollination.

In contrast, the pistil in *Mimulus guttatus* is much longer than the stamens, which themselves differ in length. The different stamen lengths may aid the visiting insects to obtain pollen. The large and sensitive bilabiate stigma opens up only when it becomes receptive.

The floral morphologies of the F_1 hybrids are somewhat intermediate between those of the parent species. Although there is a slight difference in the length of stamens and pistils, in the pollinator-free greenhouse every capsule produced by the hybrid is fully loaded with seeds. Self-pollination is facilitated by the lower lip of the stigma curling all the way back toward the anthers. Except for its autogamy, the morphology of the F_1 hybrid flower is more like that of *Mimulus guttatus*. The frequency distributions of the sum of three ratios (calyx to corolla, ovary to style and stamen to pistil) for the parent species,

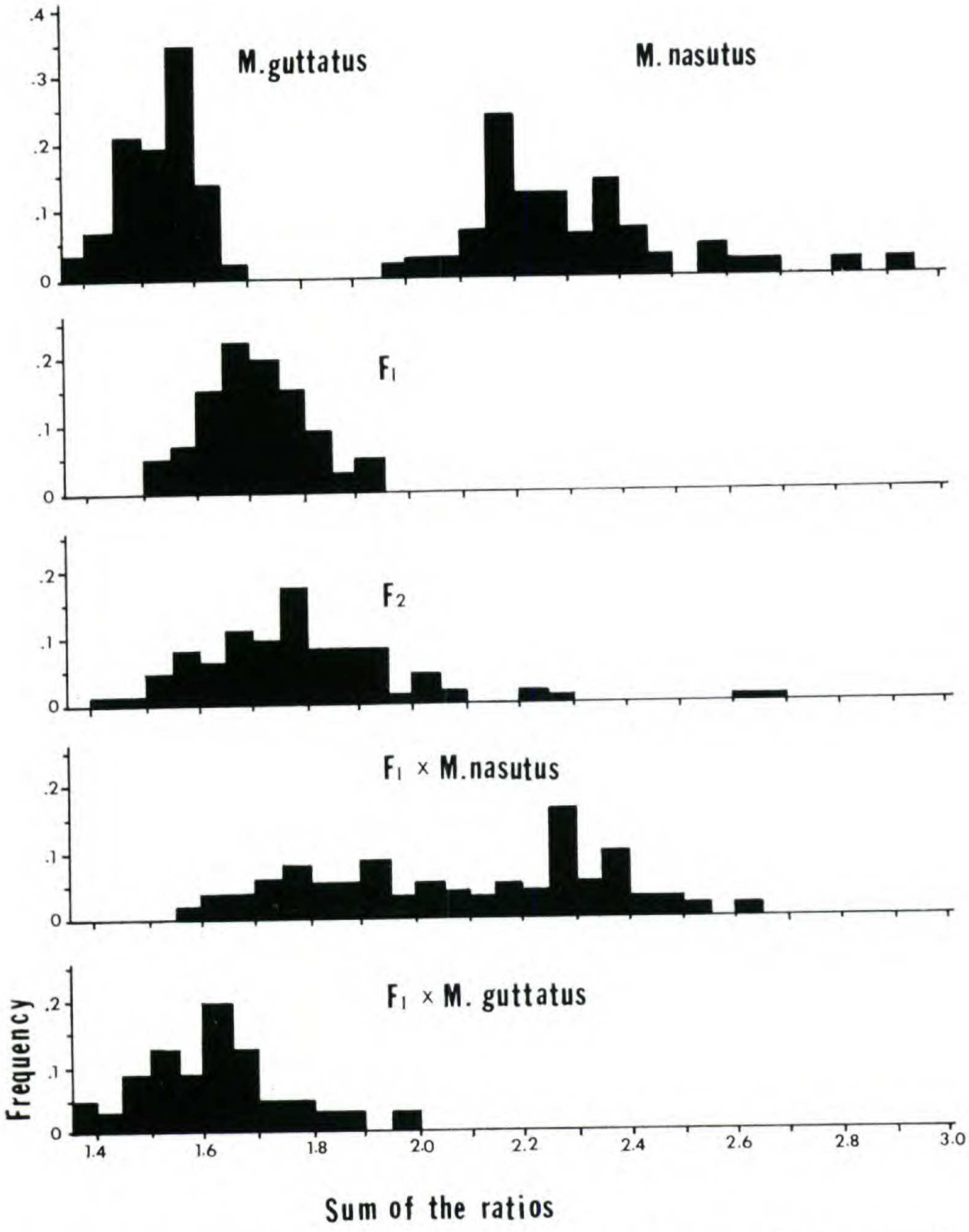

Fig. 2. Frequency distribution of the sum of ratios (calyx/corolla, ovary/style, stamen/pistil) of *M. guttatus*, *M. nasutus* and their hybrids.

the F_1, F_2 and backcross generations are plotted as histograms (Fig. 2). The distributional patterns suggest that the sum of the three ratios may be useful in studying hybridization and introgression in natural populations of *Mimulus.*

The time of anther dehiscence and occurrence of self-pollination in *Mimulus nasutus* may be a significant factor in seed setting and in reducing gene exchange between the two species. Twenty flowers each from one plant were emasculated by pulling off the corollas within 10 hours after the flowers were open. No seed was produced by the emasculated flowers of *M. guttatus.* In the case of *M. nasutus,* all emasculated flowers yielded on the average as many seeds as from non-emasculated ones. In the F_1 hybrid, 8 out of 20 emasculated flowers (or 40%) produced seed, and the average number of seed per capsule was only 49, much smaller than that produced by a non-emasculated flower.

When flower buds of the F_1 and *Mimulus nasutus* were carefully dissected to examine anthers, no pollen shedding was noticed in the F_1 hybrid, but in *M. nasutus,* the anthers had dehisced, and the stigmas were full of pollen grains. One day or two prior to the opening of the flower, the style of *M. nasutus* bends inward slightly, bringing the open stigma in contact with the dehiscent anthers to ensure self-pollination. Therefore, in *M. nasutus* pollination normally occurs before flowers open. Pseudocleistogamy has been used to describe this phenomenon (Vickery, 1964).

In annual plants, seed set in a season is one of the most crucial factors for the continuing survival of populations. Plants of *Mimulus nasutus* usually start to produce capsules when plants are still at the 2 true leaf stage. The first two or four precociously produced capsules are smaller than normal ones, and usually set seeds by cleistogamy. Several larger chasmogamous flowers very similar to those of *M. guttatus* will follow the first four flowers; then, as the blooming progresses, the flowers rapidly reduce in size.

2. Fertility and biomass

Pollen fertility.

On the average, 98% of *Mimulus nasutus* pollen and 93% of *M. guttatus* pollen were stained. The F_1 pollen (88% stained) was about 10% less fertile relative to that of *M. nasutus* and the difference is statistically significant.

Mimulus guttatus on the average produced more pollen grains per flower than *M. nasutus* (30368 ± 218 vs. 20958 ± 44). The amount of pollen produced by the F_1 hybrid (24006 ± 135) was approximately intermediate between the parental species. In *M. nasutus,* less than one-third of pollen grains remained in the anthers of newly open flowers (6775 ± 65), because pollen shedding occurs before flowers open. The precocious dehiscence of anthers in *M. nasutus* may reduce pollen flow within and between species. There is no evidence of nectar glands in *Mimulus* (Grant, 1924); thus, insects visiting the flowers are foraging for pollen. Generous pollen production would be advantageous for *M. guttatus* in achieving outcrossing.

Seed production and germination.

Mimulus guttatus produces on the average 401.5 ± 94 seeds per capsule, *M. nasutus* 256.4 ± 39 and the F_1 492. ± 96. The data indicate that the F_1 can potentially produce more seeds per capsule than the parental species.

In order to test viability, 200 seeds from each species, F_1 and the F_1 backcross were sown on moist filter paper. The germination percentages were *Mimulus guttatus,* 90.5%; *M. nasutus,* 84.0%; F_1 84.4% and F_1 backcross, 74.5%. There was no significant difference in seed viability between the parental species and the F_1 hybrid. However, the proportion of seed germination declined appreciably in the seed of the F_1 backcross.

Production of flowers, capsules and biomass.

To assess productivity, plants were harvested after growing in the greenhouse for three months. The average number of flowers, capsules, stolons, and the dry biomass

TABLE 2. Fertility and biomass production in *M. nasutus, M. guttatus*, F_1, F_2 and backcross progeny.[1]

			Main		Dry Weight (g)				
		Days to Flower	Stem Length (mm)	# Fls. & Capsules	Leaves Stems	Stolons	Fl. & Capsule	Total	Fl. & Capsule/ Total
M. nasutus N = 20	Mean	42.6	52.4	231.4	1.73	0	1.00	2.73	.366
M. guttatus N = 20	Mean	56.4	62.0	86.0	3.6	.64	.20	4.43	.045
F_1 N = 25	Mean	49.0	55.2	178.5	2.29	.22	.67	3.18	.211
F_2 N = 20	Mean	49.5	51.7	150.2	2.49	.48	.34	3.34	.102
$F_1 \times$ *M. nasutus* N = 21	Mean	48.1	47.7	181.6	1.58	.10	.69	2.37	.291
$F_1 \times$ *M. guttatus* N = 29	Mean	51.1	55.7	149.0	3.56	.31	.32	4.18	.077

[1]Plants grown in 4″ pots in the greenhouse from December 23, 1971 to March 24, 1972.

are presented in Table 2. When the productivity of the two species was compared, the differences between them were found to be statistically significant. *Mimulus nasutus* started to bloom early and terminated early. It usually branches from the base of the stem, and soon all branches become ascending with flowers.

When the reproductive effort of the plants was examined, *Mimulus nasutus* invested more energy in seed production than *M. guttatus* and the F_1. The flowers and capsules consisted of 36.6% of the total biomass in *M. nasutus*, but only 4.5% in *M. guttatus*. *Mimulus nasutus* is an annual, and seed production is crucial for continuation of the annual species. On the other hand, vegetative propagation is more important to *M. guttatus*, a perennial, for maintaining its population. In fact, *M. guttatus* showed more vegetative growth than *M. nasutus*, and produced a considerable amount of stolons, or 14.5% of the total biomass (Table 2).

In order to determine whether hybridization between the two species is generally possible, three additional populations of the two species and plants from Feather River Canyon and Eureka State Park were intercrossed. They were all interfertile.

All the experimental data indicate that barriers do not exist to prevent gene exchange either between *Mimulus guttatus* and *M. nasutus*, or between the parental species and the F_1 hybrids. The F_2 plants under greenhouse conditions were quite vigorous and showed no sign of sterility or breakdown.

3. Relationship of *Mimulus guttatus* and *M. nasutus*.

It has been well established that self-fertilized plant species are always derived from outbreeding ancestors, and annuals are most likely derived from perennials (Stebbins, 1957; Baker, 1959). The evidence from floral structure, breeding systems and fertility of hybrids strongly supports the view that *Mimulus guttatus* and *M. nasutus* are genetically closely related species and the latter is newly derived from the main species, *M. guttatus* (Vickery, 1964). It has been demonstrated with many closely related pairs of species in *Clarkia* that one member of the pair is derived from the other (Vasek, 1958; Lewis and Raven, 1958). Lewis (1962) has suggested that the daughter species in *Clarkia* are derived from marginal populations which are often subject to catastrophic selection leading to a rapid genetic reorganization and formation of the daughter species. The evolutionary relationship of *Mimulus guttatus* and *M. nasutus* may represent such a rapid evolution as a consequence of the joint action of mutation, selection and genetic drift.

Changes of breeding systems from outcrossing to self-pollination under the unusual ecological environments have been reported. *Gilia achilleaefolia* subsp. *multicaulis*, predominantly autogamous, is derived from normally outcrossing, but self-compatible *G. a.* subsp. *achilleaefolia* (Grant and Grant, 1965). A similar change has been observed in *Mirabilis longiflora*, a self-compatible species in a genus where self-incompatibility is known, which in the absence of successful insect visitation sets seeds by self-pollination (Baker, 1961a). A change of breeding system from outbreeding to inbreeding in plant species could trigger the rapid formation of a new species (Baker, 1961b). Although a direct observation of the formation of *Mimulus nasutus* from *M. guttatus* is not possible, one is tempted to reconstruct a hypothetical evolutionary process of *M. nasutus* based on the evidence gathered in this study, and on the available examples cited above.

Mimulus guttatus is a riparian species which maintains its populations largely by long life span and by growing profuse stolons as propagules in nature (Kiang and Libby, 1972). However, only in a moisture-stable environment can it grow as a perennial and reproduce vegetatively. Therefore, if environmental conditions of a peripherally isolated population happen to become dry and unstable, the population may no longer be able to maintain itself by vegetative propagation. Thus, seed production becomes a decisive factor for continuation of the population under arid conditions, which would stimulate a rapid evolution (Axelrod, 1967). Moreover, it is probable that insects may fail to visit the small number of flowers produced by one or a few plants surviving from the catastrophic selection (Lewis, 1962). Therefore, under conditions of unstable moisture availability, only individuals with successful self-pollination can produce seeds, and autogamy has obvious immediate high selective value (Stebbins, 1950; Grant & Grant, 1965).

When the environment becomes favorable, a new population may be established from the seeds produced by self-pollination. The newly founded colony with self-pollination will lead to genetic change, and those new, well adapted individuals would become the progenitors of the daughter species. Thus, the daughter species, *Mimulus nasutus* may have been derived from one or several plants which obtained a new adaptive mode under the favorable conditions.

The greenhouse and field observations strongly support the hypothesis that *Mimulus nasutus* is derived from *M. guttatus* under moisture unstable conditions. In the greenhouse 30 seeds of each species were sown in each of the 5 moisture saturated 8″ pots. One week after the seeds were sown, irrigation was completely discontinued. About 86% of *Mimulus guttatus* and 83% *M. nasutus* germinated. No *M. guttatus* survived to produce flowers, but 8 *M. nasutus* per pot survived to produce on the average 3.6 mature capsules. In nature, *Mimulus nasutus* occurs more commonly in the southern parts of the Sierra, where moisture supply is less dependable. Under normal conditions, it reaches the peak of bloom in relatively short days, April and May when moisture is most abundant (J. L. Hamrick, personal communication). The phenology of *Mimulus nasutus*, larger cotyledons with a rapid seedling growth and precociously produced capsules by self-pollination, are clearly indicative of its adaptation to a moisture unstable environment.

CONCLUSION AND SUMMARY

The breeding systems, floral structure, and phenology of *Mimulus guttatus* and *M. nasutus* were compared. The former is an outcrossing perennial while the latter is a self-pollinated annual. *Mimulus guttatus* has large, showy corollas and long pistils with sensitive labiate stigmas, whereas *M. nasutus* has small corollas which are variable in size, and short pistils with anthers clustered around stigmas. The anthers of *Mimulus nasutus* dehisce precociously and, therefore, self-pollination occurs before flowers open. Greater variation with regard to several quantitative traits was observed in *M. guttatus* than in *M. nasutus*. *Mimulus nasutus* has a higher reproductive effort than *M. guttatus,* which exhibits more vegetative growth.

Fully fertile hybrids were obtained experimentally. The growth pattern and floral characters of the F_1 hybrids are intermediate between the parental species. The hybrids can set seed by self-fertilization and grow as perennials with stolons.

A comparison of fertility and productivity indicates that there is no complete postmating isolating mechanism between *M. guttatus* and *M. nasutus.* The evidence suggests that the two species are genetically closely related and *M. nasutus* may be a daughter species newly derived from *M. guttatus. Mimulus nasutus* may represent the product of quantum evolution (Grant, 1971) as a consequence of the joint action of mutation, selection and genetic drift.

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